IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Patrizia Caldirola et al. Art Unit : 1624
Serial No. : 10/037,110 Examiner : Thomas C. McKenzie
Filed : October 22, 2001
Title : NOVEL COMPOUNDS, THEIR USE AND PREPARATION

MAIL STOP RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF PATRIZIA CALDIROLA UNDER 37 C.F.R. 1.132

1. I, Patrizia Caldirola, am a Project Leader at Biovitrum AB. Ph.D title at State University of Milan and Ph.D title at the Free University of Amsterdam in Chemistry and Pharmaceutical Sciences.

2. I and others working at Biovitrum AB conducted experiments to investigate the effect of 1-(phenylsulfonyl)-4-(1-piperazinyl)-1H-indole (disclosed as Example 7 in USSN 10/037,110 at page 23, lines 7-15 of the Specification; referred to as "Example 7" or "Applicants' Example 7 compound") and 1-[(2,5-dimethoxyphenyl)sulfonyl]-4-(1-piperazinyl)-1H-indole (disclosed as Example 8 in USSN 10/037,110 at page 23, lines 17-26 of the Specification; referred to as "Example 8" or "Applicants' Example 8 compound") on food intake in ob/ob mice. The structures of the compounds disclosed in Examples 7 and 8 are shown in Chart I. The indole ring in each of the compounds disclosed in Examples 7 and 8 is substituted at the 4-position with a piperazinyl ring.

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

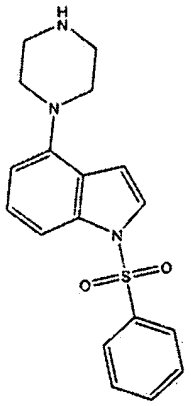
11/23/2005
Date of Deposit

A. Long
Signature

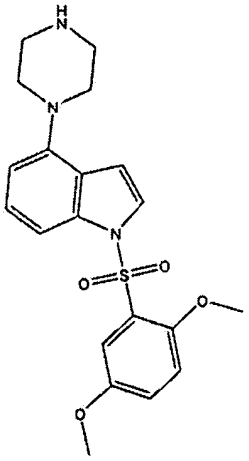
Ashley K. Long
Typed or Printed Name of Person Signing Certificate

3. I and others working at Biovitrum AB also conducted separate experiments to investigate the effect of the 5-HT6 antagonist "compound **4a**," which is disclosed in Isaac, M. et al., *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1719-1721 (Isaac) on food intake in ob/ob mice. The structure of compound **4a** is also shown in Chart I. The indole ring in compound **4a** is substituted at the 6-position with a bicyclopiperazinyl ring. The binding constant (K_i (5-HT_6)) for compound **4a** is disclosed in Isaac to be 0.2 nM. Compound **4a** has the lowest Ki of all the compounds tested in Isaac.

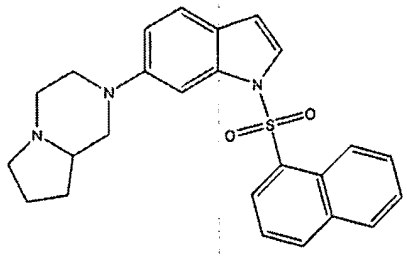
Chart I



Example 7



Example 8



Compound 4a

Effect of 1-(phenylsulfonyl)-4-(1-piperazinyl)-1H-indole (Example 7), 1-[(2,5-dimethoxyphenyl)sulfonyl]-4-(1-piperazinyl)-1H-indole (Example 8), and compound 4a (referred to collectively as "the test compounds") on Food Intake

4. The effect of each of the test compounds on food intake was determined as follows.

Applicants' Example 7

I. Obese ob/ob mice were divided into four groups, referred to as "Groups 7-A, 7-B, 7-C, and 7-D." Each of the aforementioned groups contained 8 mice.

Group 7-A was treated with vehicle only (saline) on the day of treatment and thus served as a control group. Groups 7-B and 7-C were each treated with different dosages of Applicants' Example 7 compound on the day of treatment: each of the 8 mice in Group 7-B was dosed at 50 mg/Kg/day, and each of the 8 mice in Group 7-C was dosed at 150 mg/Kg/day. Finally, Group 7-D was treated with *m*-chlorophenylpiperazine (*m*CPP, a known 5-HT_{2c} agonist) on the day of treatment and thus served as a positive control group.

II. Obese ob/ob mice were divided into five groups, referred to as "Groups 7-A', 7-B', 7-C', 7-D', and 7E'." Each of the aforementioned groups contained 8 mice.

Group 7-A' was treated with vehicle only (saline) on the day of treatment and thus served as a control group. Groups 7-B', 7-C', 7-D', and 7E' were each treated with different dosages of Applicants' Example 7 compound on the day of treatment: each of the 8 mice in Group 7-B' was dosed at 3 mg/Kg/day; each of the 8 mice in Group 7-C' was dosed at 10 mg/Kg/day; each of the 8 mice in Group 7-D' was dosed at 30 mg/Kg/day; and each of the 8 mice in Group 7-E' was dosed at 50 mg/Kg/day.

Applicants' Example 8

Obese ob/ob mice were divided into five groups, referred to as "Groups 8-A, 8-B, 8-C, 8-D, 8-E." Each of the aforementioned groups contained 8 mice.

Groups 8-A was treated with vehicle only (saline) on the day of treatment and thus served as a control group. Each of the 8 mice in Group 8-B was dosed with 3 mg/Kg/day of Applicants' Example 8 compound; each of the 8 mice in Group 8-C was dosed with 10 mg/Kg/day of Applicants' Example 8 compound, and each of the 8 mice in Group 8-D was dosed with 30 mg/Kg/day of Applicants' Example 8 compound. Finally, Group 8-E was treated with (*m*CPP) on the day of treatment and thus served as a positive control group.

Isaac's Compound **4a**

Obese ob/ob mice were divided into five groups, referred to as "Groups **4a**-A, **4a**-B, **4a**-C, **4a**-D, **4a**-E." Again, each of the aforementioned groups contained 8 mice.

Group **4a**-A was each treated with vehicle only (saline) on the day of treatment and thus served as a control group. Each of the 8 mice in Group **4a**-B was dosed with 10 mg/Kg/day of Isaac's Compound **4a**; each of the 8 mice in Group **4a**-B was dosed with 30 mg/Kg/day of Isaac's Compound **4a**; and each of the 8 mice in Group **4a**-B was dosed with 100 mg/Kg/day of Isaac's Compound **4a**. Finally, Group **4a**-E was treated with (*m*CPP) on the day of treatment and thus served as a positive control group.

In all of the above-mentioned experiments, administration of the test compounds; *m*CPP, a non-selective 5HT2c agonist as a positive control; and vehicle was conducted using osmotic mini-pumps (Alzet Model 20001D; rate of 8ul/h) that were subcutaneously implanted into the animals under short-acting anaesthesia (according to Alzet technical information manual).

The minipumps were either filled with different concentrations of the test compounds dissolved in vehicle or with only vehicle and kept in solution for at least 1 hour at 37 C. The in vivo efficacy of test compounds was investigated by recording the amount of food consumed during a period of 15 hours in obese C57BL/6J-ob/ob male mice (Bomholtsgaard).

To obtain baseline values food intake was recorded at 8 a.m. and at 17 p.m. for two days before the administration of test compounds. T he amount of food consumed between 17 p.m. and 8 a.m. (15 hours) was used for calculations. *m*CPP (30 mg/kg/day), a non-selective 5HT2c agonist, was used as a reference drug in all but experiment **II** under "Applicants' Example 7."

Food intake data is shown in two different formats in Tables 1, 2, 3, and 4 below. "Mean Pre-treatment (g/15h)" for a particular group refers to the mean weight (g = grams) of food consumed per mouse over a 15 hour period on the day before treatment (basal). "Mean Post-treatment (g/15h)" for a particular group refers to the mean weight (grams) of food consumed per mouse over a 15 hour period after treatment with the test compound. The term "% of basal level" refers to quotient of:

Mean Post-treatment (g/15h)/Mean Pre-treatment (g/15h)

For example, if a particular group consumed on average 6.0 g of food per animal over a 15 hour period on the day before treatment (basal) and then consumed only 1.5 g of food over a 15 hour period after treatment with the test compound, then the "% of basal level" for that particular group would be 25% (i.e., the animal's food consumption after treatment was only 25% of that observed on the day before treatment). A "% of basal level" of less than 100% means that post-treatment food consumption was lower than pre-treatment food consumption). The lower the "% of basal level" (both in absolute value and relative to the % of basal level of a control (i.e., untreated) group), the more effective the test compound is at reducing food intake.

5. Applicants' Example 7 Compound: 1-(phenylsulfonyl)-4-(1-piperazinyl)-1H-indole reduced food intake in the obese ob/ob mouse model.

The data for Example 7-I is summarized in Table 1.

Table 1

Compound/ (Group)	Dosage (mg/kg/day)	Mean Pre-treatment (g/15h)	Mean Post-treatment (g/15h)	% of basal level
Vehicle (saline)/ (7-A)	-	5.7	4.3	77
Example 7/ (7-B)	50	5.4	2.1	39
Example 7/ (7-C)	130	4.8	0.8	16
*m*CPP/(7-D)	30	5.2	2.1	41

The greatest reduction in food intake was observed in Group 7-C (% of basal level = 16%), in which Applicants' Example 7 compound was administered at a dosage of 130 mg/kg/day. The % of basal level for Group 7-C (16%) was significantly lower than that observed for the control group 7-A (77%). In fact, the % of basal level for Group 7-C was the lowest observed in any of the experiments described herein.

Reduction in food intake was also observed in Group 7-B (% of basal level = 39%), in which Applicants' Example 7 compound was administered at a dosage of 50 mg/kg/day. The % of basal level for Group 7-B (39%) was lower than that observed for the control group (77%) and was comparable to that observed in the positive control group 7-D (41%) in which *m*CPP was administered at 30 mg/kg/day.

The data for Example 7-II is summarized in Table 2.

Table 2[1]

Compound/ (Group)	Dosage (mg/kg/day)	Mean Pre-treatment (g/15h)	Mean Post-treatment (g/15h)	% Inhibiton of basal level
Vehicle (saline)/ (7-A')	-	5.7	4.2	73
Example 7/ (7-B')	3	5.8	3.7	63
Example 7/ (7-C')	10	4.9	3.0	53
Example 7/ (7-D')	30	5.5	1.7	33
Example 7/ (7-E')	50	6.3	1.5	25

The greatest reduction in food intake was observed in Group 7-E' (% of basal level = 25%), in which Applicants' Example 7 compound was administered at a dosage of 50 mg/kg/day. The % of basal level for Group 7-E' (25%) was significantly lower than that observed for the control group 7-A (73%).

Reduction in food intake was also observed in Group 7-D' (% of basal level = 33%), in which Applicants' Example 7 compound was administered at a dosage of 30 mg/kg/day. The % of basal level for Group 7-D' (33%) was significantly lower than that observed for the control group (73%).

Reduction in food intake was also observed in Group 7-C' (% of basal level = 53%), in which Applicants' Example 7 compound was administered at a dosage of 10 mg/kg/day. The % of basal level for Group 7-C' (53%) was lower than that observed for the control group (73%).

Finally, statistically meaningful reduction in food intake was even observed at relatively low dosages (3 mg/kg/day) of Applicants' Example 7 compound (see data for Group 7-B').

---

[1] Note that the formulation consists of 50% PEG 400 and 50 % tween 80.

6. Applicants' Example 8 Compound: 1-[(2,5-dimethoxyphenyl)sulfonyl]-4-(1-piperazinyl)-1H-indole (Example 8) also reduced food intake in the obese ob/ob mouse model.

The data for Example 8 is summarized in Table 3.

Table 3

Compound/ Group	Dosage (mg/kg/day)	Mean Pre-treatment (g/15h)	Mean Post-treatment (g/15h)	% of basal level
Vehicle (saline)/ (8-A)	-	6.11	4.19	68.0
Example 8/ (8-B)	3	5.96	3.67	62.8
Example 8/ (8-C)	10	5.87	3.10	53.4
Example 8/ (8-D)	30	5.95	1.89	32.8
*m*CPP/(8-E)	10	5.71	2.57	45.9

For Applicants' Example 8, the greatest reduction in food intake was observed in Group 8-D (% of basal level = 32.8%), in which Applicants' Example 8 was administered at a dosage of 30 mg/kg/day. The % of basal level for Group 8-D (32.8%) was significantly lower than that observed for the control group 8-A (68.0%).

Reduction in food intake was also observed in Group 8-C (% of basal level = 53.4%), in which Applicants' Example 8 was administered at a dosage of 10 mg/kg/day. The % of basal level for Group 8-C (53.4%) was lower than that observed for the control group and was comparable to that observed in the positive control group 8-E (45.9%) in which *m*CPP was also administered at 10 mg/kg/day.

Finally, statistically meaningful reduction in food intake was even observed at relatively low dosages (3 mg/kg/day) of Applicants' Example 8 (see data for Group 8-B).

7. Compound **4a**: Compound **4a** was not effective in reducing food intake in the obese ob/ob mouse model.

The data for Compound **4a** is summarized in Table 4.

Table 4

Compound/ Group	Dosage (mg/kg/day)	Mean Pre-treatment (g/15h)	Mean Post-treatment (g/15h)	% of basal level
Vehicle (saline)/ (**4a**-A)	-	5.65	4.19	75.21
Compound **4a**/ (**4a**-B)	10	5.88	4.58	79.55
Compound **4a**/ (**4a**-C)	30	5.89	4.34	75.84
Compound **4a**/ (**4a**-D)	100	5.83	4.31	74.39
*m*CPP/(**4a**-E)	10	6.00	2.85	48.34

The % of basal level for Group **4a**-B (79.55%) was actually slightly higher than the % of basal level for the control group **4a**-A (75.21%). This means that there was effectively no reduction in food intake when the animals were dosed with 10 mg/kg/day of Isaac's compound **4a**.

In contrast, when the animals were dosed with 10 mg/kg/day of Applicants' Example 7 compound (see data and discussion for Group 7-C'), the % of basal level for Group 7-C' (53%) was lower than that observed for the control group (73%). Thus, food intake was lowered in Group 7-C' (10 mg/kg/day of Applicants' Example 7 compound), but not in Group **4a**-B (10 mg/kg/day of Isaac's compound **4a**).

Again, in contrast with Isaac's compound **4a**, when the animals were dosed with 10 mg/kg/day of Applicants' Example 8 compound (see data and discussion for Group 8-C), the % of basal level for Group 8-C (53.4%) was lower than that observed for the control group (68.0%) and was comparable to that observed in the positive control group 8-E (45.9%). Thus, food

intake was lowered in Group 8-C (10 mg/kg/day of Applicants' Example 8 compound), but not in Group **4a**-B (10 mg/kg/day of Isaac's compound **4a**).

The % of basal level for Group **4a**-C was essentially the same as the % of basal level for the control group **4a**-A. This means that there was effectively no reduction in food intake when the animals were dosed with 30 mg/kg/day of Isaac's compound **4a**.

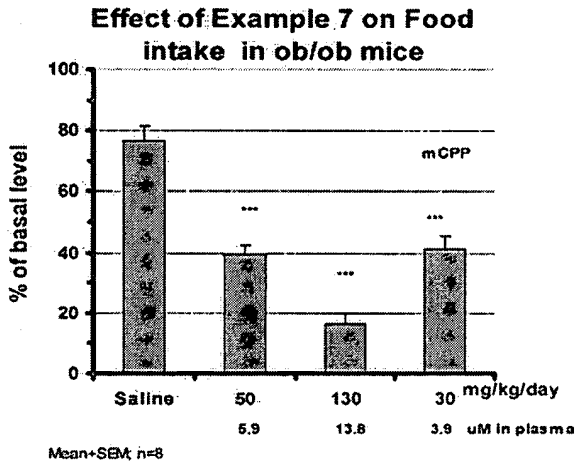
In contrast, when the animals were dosed with 30 mg/kg/day of Applicants' Example 7 compound (see data and discussion for Group 7-D'), the % of basal level for Group 7-D' (33%) was lower than that observed for the control group (73%). Thus, food intake was lowered in Group 7-D' (30 mg/kg/day of Applicants' Example 7 compound), but not in Group **4a**-C (30 mg/kg/day of Isaac's compound **4a**).

Again, in contrast with Isaac's compound **4a**, when the animals were dosed with 30 mg/kg/day of Applicants' Example 8 compound (see data and discussion for Group 8-D), the % of basal level for Group 8-D (32.8%) was lower than that observed for the control group (68.0%). Thus, food intake was lowered in Group 8-D (30 mg/kg/day of Applicants' Example 8), but not in Group **4a**-C (30 mg/kg/day of Isaac's compound **4a**).

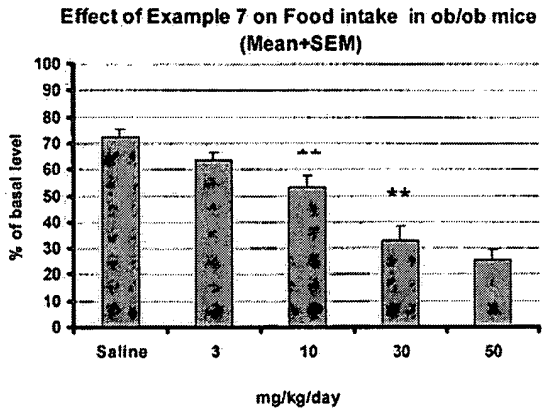
Finally, even at a relatively high doses, Isaac's compound **4a** was not effective at reducing food intake (see data and discussion for Group **4a**-D, in which the animals were dosed with 100 mg/kg/day of Isaac's compound **4a**). In contrast, when the animals received a relatively high dose of Applicants' Example 7 (see data and discussion for Group 7-C), the % of basal level for Group 7-C (16%) was significantly lower than that observed for the control group 7-A (77%) and was the lowest observed in any of the experiments described herein. Thus, food intake was lowered in Group 7-C (130 mg/kg/day of Applicants' Example 7), but not in Group **4a**-D (100 mg/kg/day of Isaac's compound **4a**), despite the use of a relatively high dose of Isaac's compound **4a**.

8. Shown below are graphical summaries of the "% of basal level" observations made in the experiments described herein:

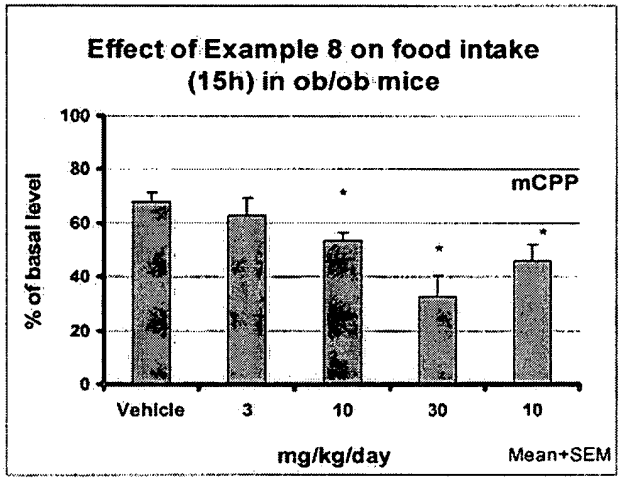
Example 7-I:



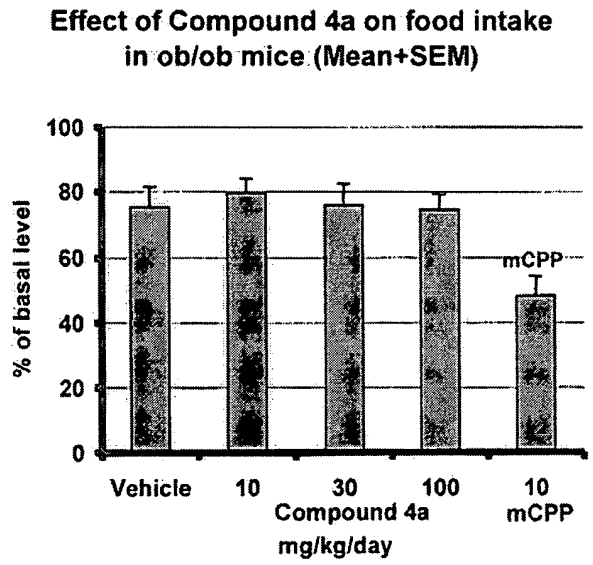
Example 7-II:



Example 8:



Compound 4a:



9. Isaac's compound **4a**, although a 5-HT_6 antagonist (as well as the most potent 5-HT_6 antagonist disclosed in Isaac), was not effective at reducing food intake in the obese ob/ob mouse model at any dosage. On the other hand, Applicants' Example 7 and Example 8 were indeed effective at reducing food intake in the obese ob/ob mouse model over a relatively wide range of dosages. Thus, on the basis of the results obtained with the foregoing animal model, Applicants' Example 7 and Example 8 possess the ability to reduce food intake, while Isaac's compound **4a** does not.

10. I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the instant patent application or any patent issued thereon.

Date: 23 November 2005 Patrizia Caldirola

Patrizia Caldirola